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Efficacy in Microbial Sterilization of Pulsed Magnetic Field Treatment

Ma Haile* Zhongli Pan†

Mengxiang Gao‡ Lin Luo**

*Jiangsu University & Henan University of Science & Technology, mhl@ujs.edu.cn

†University of California, Davis & United States Department of Agriculture, zlpan@ucdavis.edu

‡Yangtze University, mxgao0398@sohu.com

**Jiangsu University, luolin@ujs.edu.cn

Efficacy in Microbial Sterilization of Pulsed Magnetic Field Treatment*

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Abstract

Sterilization effects of the pulsed magnetic field with a maximum intensity of 11.37 Tesla were investigated on Escherichia coli AS 1.129, Staphylococcus aureus AS 1.89, Saccharomyces cerevisiae ATTC 7552 and Bacillus subtilis AS 1.921. The well-regulated fluctuations of sterilization effects with magnetic field intensity and pulse number were observed, and can be described by the "window effect" of magnetic fields and provide a better explanation of the inconsistent results of PMF sterilization in published literature. Sensibility of bacteria on the pulsed magnetic field significantly depends on a variety of microorganisms. Sterilization effects of a flowing sample were better than that of static samples.

KEYWORDS: magnetic field, pulse, sterilization, window effect

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1 Introduction

Non-thermal sterilization methods have been great research interests in processing various products including foods, drugs and bio-products to overcome the adverse quality changes caused by traditional thermal sterilizations. In recent years, the most researches and applications of the non-thermal sterilizations focused on high hydrostatic pressure (HHP) processing (Kalchayanand, Sikes, & Dunne, 1998; Garcia-Graells, Hauben, & Michiels, 1998; Aleman, Ting, & Mordre, 1996; Berlin, Herson, & Hicks, 1999). Other non-thermal methods, including pulsed magnetic fields (PMF), oscillating magnetic fields (OMF), pulsed electric fields (PEF), ultrasound (US), pulsed light (PL), ultraviolet (UV) light and pulsed X-rays (PXR), have also been studied as alternative food processing technologies (Frank, Ashim, & Jozef, 2000; San Martin, Harte, & Lelieveld, 2001).

The researches on the effect of the high intensity PMF on microbial activity were scarce and the results were controversial. Hofman (1985) used an oscillating magnetic field to produce PMF and found that the PMF was very effective in deactivation of microorganisms in foods. In the study, the food was kept in a sealed plastic bag and treated for various times from 25 to 100 ms at the temperature of 0 to 50°C using 1 to 100 pulses of PMF at a frequency between 5 to 500 kHz. However, it has also been reported that the PMF treatment had no significant effect on microbial populations (San Martin, Harte, & Huub Lelieveld, 2001; Caubet, 1999; Malko, Constantinidis, & Dillehay, 1994) and even enhanced the microbial growth (Okuno, Tuchiya, Ano, & Shoda, 1993). The study of PMF parameters on sterilization effect and mechanism is very limited. The available research information is not enough to explain the controversial results. Also, static PMF treatment was typically used in the most of the study. In such treatment, because the magnetic field at the sterilization chamber inside the coil was not uniform, uniform sterilization in the test products may not achieved. However, if the product is under dynamic condition, the microorganisms in the product may have increased opportunity to be uniformly exposed to magnetic field and improve inactivation efficiency. Chen Guozhang and Chen Xiaohui (1998) reported that the non-thermal biological effect of magnetic fields with low intensity and frequency (Hz) could impact the sterilization results. Therefore, it is important to determine the effect of PMF parameters under static and dynamic conditions on sterilization of various microbial.

Our group has systematically researched the effects of PMF treatment for

sterilization. The studied PMF parameters included magnetic field factors, such as intensity and pulses (Yang Qiaorong, Gao Mengxiang, & Ma Haile, 2004), product properties, such as ionic concentration, temperature and pH (Ma Haile, Gao Mengxiang, & Guo Kangquan, 2004), and physiological factors, such as microbial cell concentration. The sterilization effect of PMF treatment on bacteria at different population growth stages and the change and distribution in temperature of model food liquid containing bacteria in the sterilization chamber were also measured (Ma Haile, Wu Qiongying, Gao Mengxiang, & Chu Jinyu, 2004). The treated materials included model liquid containing *E. coli*, *S. aureus*, *S. cerevisiae* and *B. subtilis* (Ma Haile, Gao Mengxiang, & Guo Kangquan, 2004; Cao Hui, Ma Haile, Cui Henglin, & Liu Tao, 2003), milk (Gao Mengxiang, Ma Haile, & Guo Kangquan, 2005), foremilk (Luo Xinzheng, & Ma Haile, 2004), watermelon juice (Gao Mengxiang, Ma Haile, & Guo Kangquan, 2004; Ma Haile, Deng Yulin, & Chu Jinyu, 2003) and beer (Ma Haile, Deng Yulin, & Chu Jinyu, 2003). These studies provided us fundamental information about the effectiveness of PMF treatment for sterilization, which made us believe that the state of treated materials and intensity of PMF could be important factors in using the non-thermal treatment method.

Objective of this study was to investigate effect of the PMF parameters on the sterilization of various bacterial under static and dynamic PMF treatments.

2 Materials and Methods

2.1 Microorganism

This research studied the responses of four different microorganisms to different PMF treatments. The used microorganisms are the strains of *Escherichia coli* AS 1.129 (*E. coil*), *Staphylococcus aureus* AS 1.89 (*S. aureus*), *Saccharomyces cerevisiae* ATTC 7552 (*S. cerevisiae*) and *Bacillus subtilis* AS 1.921 (*B. subtilis*) obtained from China General Microbiological Culture Collection Center (CGMCC).

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2.2 Sample reparation

(1) Preparation of microorganism nutrient mediums

Three nutrient mediums were prepared and used for specific organisms. The nutrient medium preparation procedures and ingredients are described below.

The nutrient mediums for *E. coli* and *S. aureus* consisted of 10g of peptone, 5g of beef paste, 5g of NaCl, 20g of agar and 1000ml of distillated water. Except agar, all ingredients were dissolved into the distillated water, then two milliliters of 15% NaOH solution were added into the solution for achieving the desired pH of 7.2-7.4. As the last step, agar was added into the solution, followed by heating to obtain agar gel. The obtained nutrient mediums were packed into a 500mL flask and sterilized at 121 °C for 20 min before they are used.

The nutrient medium for *S. cerevisiae* consisted of 10g of yeast extract, 10g of peptone, 20g of glucose, 20g of agar and 1000ml of distillated water. The preparation procedure was the same as that of nutrients for *E. coli* and *S. aureus*, except for the pH value of the nutrient medium was 6.5.

The nutrient medium for *B. subtilis* was also prepared with the same procedure with different ingredients and had final pH valve of 7.2-7.4. The ingredients included 10g of beef paste, 10g of peptone, 3g of K_2HPO_4, 5g of NaCl, 0.03g of $MgSO_4$, 20g of agar and 1000ml of distillated water.

(2) Culture of microorganisms

The small amount of microorganisms preserved in test tubes under refrigeration condition was selected for inoculation with corresponding bevel nutrient mediums. The culture temperatures and times were 37±1°C for *E. coli, S. aureus* and *S. cerevisiae*, 28±1°C for *S. cerevisiae,* 45±1°C for *B. subtilis* for 24 h. Then they were inoculated in their flat nutrient mediums under germfree condition and cultured at same temperature with bevel culture for additional 24 h except for *B. subtilis* which had 48 h.

(3) Preparation of sterilization samples

The samples for sterilization test were prepared from elution liquid which was obtained by eluting the bacteria from flat nutrient medium with sterile 0.9%

isotonic saline. The elution liquid was aspirated and putted into a sterile triangle flask for further dilution using 0.9% isotonic saline to the desired concentration. During the dilution the sample was mixed to achieve uniformity. The liquid suspension with bacteria was placed into sterile sample tubes and stored in liquid nitrogen until use in sterilization test.

(4) Microbial enumeration

The numbers of microbial viable cells before and after PEF treatment were determined with the total plate count method using a nutritional growth medium. The 10-fold serial dilutions were achieved with 0.85% NaCl solution. Duplicates of each dilution were made. The sterilization effect was calculated using the survival rate N/N_0. N and N_0 represented total bacterial counts of before and after-sterilization, respectively.

2.3 Sterilization method of using the pulsed magnetic field

The sterilization experiments were carried out using the system designed by Jiangsu University (Jiangsu, Zhenjiang, China). The PMF was generated by automatically alternating the charge and discharge to coil in the chamber using a series of capacitances. Maximum magnetic field intensity generated by this equipment was 11.37 Tesla (T).

The sterilization tests were conducted in two groups based on the samples holders, including glass vial and plastic tube. Tests using glass vial and plastic tube will be called respectively vial-test and tube-test hereinafter. Vial-test is to study the basic biological effect of the pulsed magnetic field. Tube-test is for future industrialized application of this technology. For the tube-test, samples in tube have two statuses, with or without flowing, and the corresponding tests will be called static-tube-test and dynamic-tube-test. The design on two statuses is to compare the sterilization effect of static test and dynamic test.

For the vial-test, the sample holder is a sterile glass vial with 18 mm internal diameter × 30 mm length. Before the samples were put into the sample holders, 1ml of thawed bacteria at room temperature was diluted using sterile 0.9% isotonic saline to ratio of 1:10. Then 5 ml of diluted sample was placed into the glass vial and sealed. The sample vial was set at the columnar sterilization chamber center and treated at room temperature. The test parameters were magnetic field intensity

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from 1.21 to 9.48T and pulse number from 5 to 35 at room temperature (18±1°C).

For the tube-test, the sample holder is a straight soft plastic tube with 5mm of internal diameter, being placed along axes of columnar sterilization chamber. The amount of the sample of static-tube-test was 8 ml for each batch. The test parameters for the static-tube-test were magnetic field intensity from 2.11 to 3.79T and pulse number from 10 to 50 at room temperature (10±1°C) (20 pulses). The sample flowed through the chamber with 3.37T of PMF intensity at room temperature (6±1°C) for the dynamic-tube-test at the flow rate from 4 to 32ml/min. Microorganism for the dynamic sterilization tests is *S. cerevisiae*.

3. Results and discussion

3.1 Effect of PMF intensity and pulse number on sterilization for vial-tests

Sterilization tests results from the vital tests (Figure 1) clearly showed that the survival rate of bacteria varied with the PMF intensity. Under the low intensity, the survival rates decreased rapidly and then reached minimum. However, by further increasing the intensity, the survival rate increased again and at the maximum intensity tested it decreased again. The results revealed the effectiveness in sterilization of PMF treatment is closely related to the intensity and species of the bacteria. *S. cerevisiae* reached to minimum value 6.7% at 5.07T. At 6.33T the lowest survival rates of *E. coli*, *S. aureus* and *B. subtilis* were achieved with minimum values of 2.25%, 3.8% and 22.5%, respectively. The reasons causing the different response to the PMF treatment were not clear and needs to be further studied for revealing the PMF sterilization mechanism.

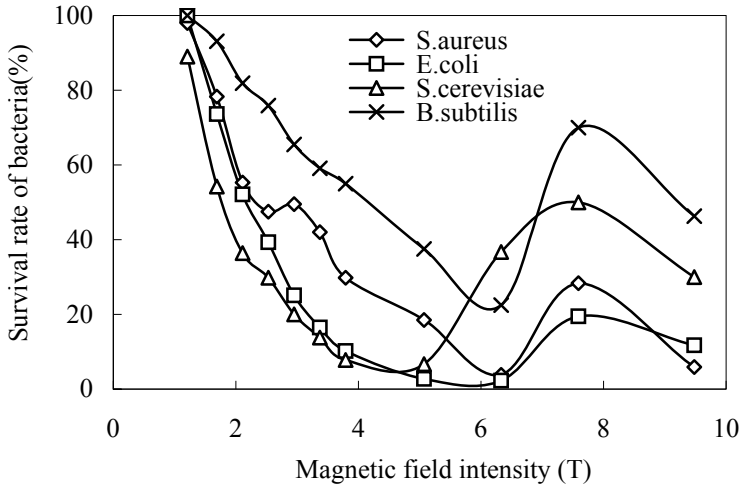

Figure 1 Effect of magnetic field intensity on survival rate of bacterias (vial-tests, 20 pulses)

The effects of pulse number on sterilization are shown in Figure 2 and Figure 3 for vial-tests. It can be seen from Figure 2 under 3.37T of PMF intensity that bacterial survival rate generally decreased with the increase of the number of pulses. The minimum survival rates of *E. coli*, *S. aureus* and *S. cerevisiae* appeared at 30, 25 and 20 of pulse numbers, respectively, and their minimum values were similar from 10.2% to 13.3%. However, by further increasing the numbers of pulses could make the treatment less effective. When the PMF intensity increased from 3.37T to 6.33T, the trend of bacteria survival rates was also changed (Figure 3). At high numbers of intensity, very high bacteria survival rates were received. The minimum bacteria rates appeared in the range of 10-20 of pulse numbers. It seems that different bacteria responded to the treatment differently. The most effective treatment results were obtained for *S. aureus* and *S. cerevisiae*.

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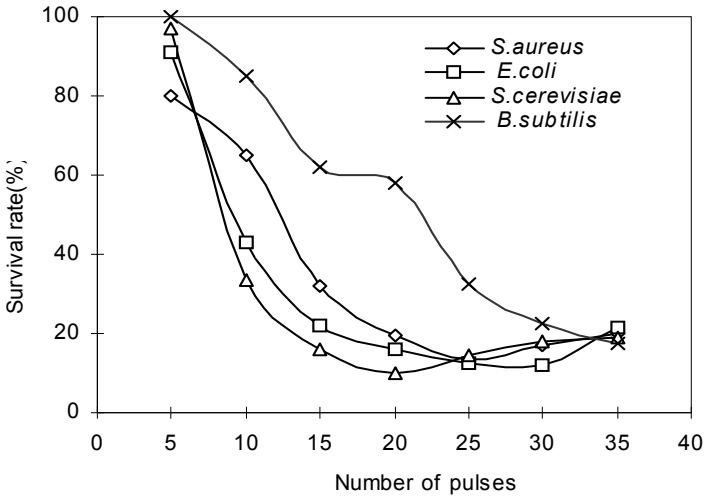

Figure 2 Effect of number of pulses on survival rate of bacteria (vial-tests, 3.37T)

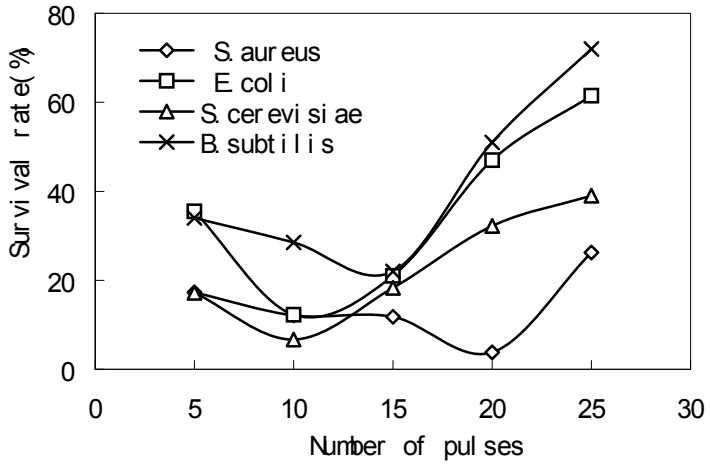

Figure 3 Effect of number of pulses on survival rate of bacteria (vial-tests, 6.33T)

3.2 Effect of PMF intensity and pulse number on sterilization for static-tube-tests

Batch tests (Figure 4) showed that PMF intensity corresponding to the minimum survival rate 11.1% for *E. coli* and 2.6% for *S. aureus* was 3.37T. Survival rate of *S. cerevisiae* always kept lower level under the PMF intensity from 2.53T to 3.37T,

the value was from 2.5% to 1.2%. After the best PMF intensity, bacterial survival rate, being similar to vial-tests, increased gently. The order of minimum bacterial survival rate was *S. cerevisiae*, *S. aureus* and *E. coli*. Simple reason why sterilization effect of batch-tests was better than vial-tests was that thin tube of sample in batch-tests was just centralized near the axis of cylindrical chamber, where the line of magnetic force was denseness. However the sample vials of vial-tests were placed at wider field along the direction of diameter of cylindrical chamber.

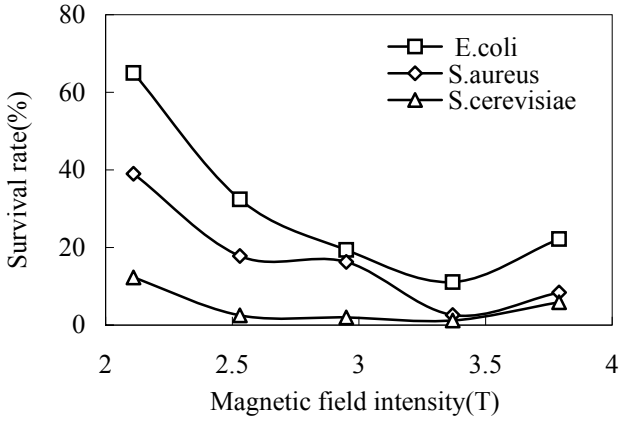

Figure4 Effect of magnetic field intensity on survival rate of bacteria (static-tube-tests, 20 pulses)

Beside similar trends for batch tests (Figure 5), another important phenomenon was observed, which was that the vale-values of bacterial survival rate appeared, repetitively.

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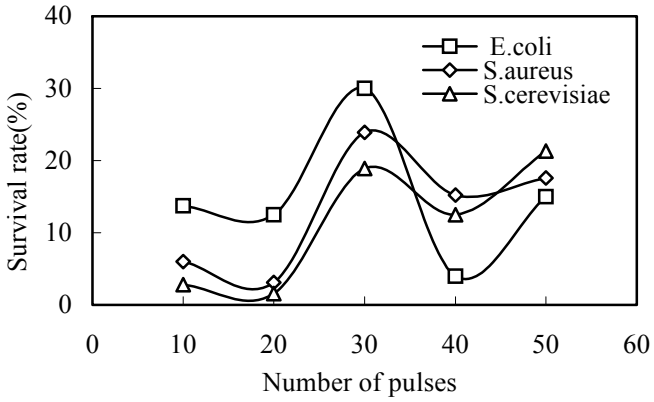

Figure 5 Effect of number of pulses on survival rate of bacteria (batch-tests, 3.37T)

Reviewing the results from Figure 1 to Figure 5, some common conclusions can be obtained and be discussed as follows:

Firstly, vale-values of bacterial survival rate appeared almost in every sterilization test group of this research, in despite of the effects from PMF intensity or from pulse number. According to the general electromagnetic theory, to the pulsed magnetic field, the increase of magnetic field intensity result in increased change of magnetic flux ($d\varphi/dt$) crossing cell membrane of bacteria and damage of cell membrane. According to the electroporation of cell membrane (Li Jixi, & Niu Zhongqi, 1990), when voltage applied on cell membrane is higher than a critical value, penetrability of cell membrane increases quickly, resulting in many small holes on cell membrane and reduced strength of cell membrane. High magnetic field intensity can also induce high electronic field intensity, which should result in more significant destruction of bacterial cell membrane. According to definition of Lorentz force, high magnetic field intensity corresponds to small movement radius of electriferous particles. So when magnetic field intensity reaches a critical value, electriferous particles will be closed inside bacterial cell and circumgyrate repeatedly, nutrients outside and wastes inside cell membrane can not be exchanged (Zhang Xiaoyun, 1989). Therefore, the increased damage of bacteria could be achieved when the magnetic field intensity increases to certain extent. According classic theory on electromagnetic, electroporation of cell membrane and Lorentz force, bacterial survival rate decreases monotonously with magnetic field intensity, so the obtained results can not be explained by the classic theory. The effect of the number of pulse on bacterial survival was unexpected because intense treatment

may not resulted in low bacterial survival.

The Bawin and Adey's studied (1978) non-thermal biological effect of electromagnetic wave with extremely low frequency and low intensity pointed that "window effect" is one of very important characteristics of electromagnetic field. The "window effect" indicates that targets inside bio-system only respond to the electromagnetic waves with some discrete frequency or intensity range, so it are called "Frequency Window" and "Intensity (or Power Density) Window". Blankman, et al (1998) repeated experiment of Bawin and Adey, and found that "Frequency Window" had series of values but only one. The research of Byus (1984) found "Time Window". The obtained results with PMF in this study could be explained by the window effect found in electromagnetic treatment. Figure 7 shows that "Time Window" appears repeatedly. It could be found from Figure 3 that "Intensity Window" has a trend of the repeated appearance.

The "vale value" or "window effect" is an important phenomenon of bacteria inactivation using the pulsed magnetic field with high intensity. It has happened in the sterilization tests of some food, such as watermelon juice (Gao Mengxiang, Ma Haile, & Guo Kangquan, 2004; Ma Haile, Deng Yulin, & Chu Jinyu, 2003), milk (Gao Mengxiang, Ma Haile, & Guo Kangquan, 2005).

Perhaps "window effect" can provide a good explain for research results from Harte, F. M. (2001) who found that no additional inactivation or cell damage for *E. coli* due to exposure to the pulsed magnetic field (50 pulses, 18 T) at 42°C and Caubet (1999) who observed that *Listeria innocua*, *E. coli* and *Bacillus cereus* exposed to 1-6 pulses of a 7-T MF did not affect significantly their growth parameters. One possible reason is that the parameters 50 pulses/18 T for *E. coli* and 1-6 pulses/7T for *Listeria innocua*, *E. coli* and *Bacillus cereus* appeared outside "Time Window" and "Intensity Window", respectively.

Secondly, different microorganism has different sensitivity to the pulsed magnetic field. It could be found from Figure1 to Figure 5 that *B. subtilis* was most difficult to inactivate and *S. cerevisiae* was easiest to inactivate in four bacteria. The minimum survival rates of *B. subtilis* were the highest (Fig.1 and Fig.3) and curves of survival rate of *B. subtilis* were over other 3 bacteria under most cases (Fig.1, Fig.2 and Fig.3). The minimum survival rates of *S. cerevisiae* were or closed to the lowest (Fig.1 to Fig.5). The curves of survival rate of *S. cerevisiae* located almost at the lowest position (Fig.1 to Fig.5). The reason why *B. subtilis* is difficult to inactivate is that cell of *B. subtilis* has a hard crust.

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3.3 Effect of flow rate of sample on sterilization for dynamic-tube-tests

The effects of sample flow rate on the survival of *S. cerevisiae* are shown in Figure 6. The flow rate did not show significant effect on the bacteria survival when the flow rate was less than 16 ml/min. By further increasing the flow rate, the survival rate reduced quickly to minimum of 0.8% at the flow rate of 26 ml/min. However, when the flow rate reached to 32 ml/min, the survival rate increased again. This indicated that the survival rate was closely related to the flow rate.

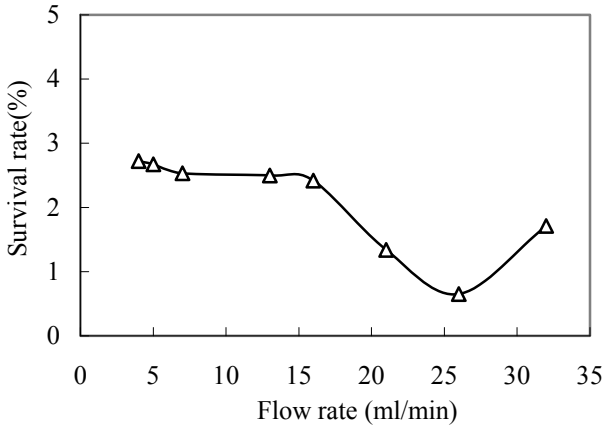

Figure 6 Effect of velocity of flow on survival rate of S. cerevisiae (dynamic-tube-tests, 3.37T)

Influence of sample flow rate on sterilization effect is complicated. Although lower flow rate at first 16 minutes could stand sample longer time for exposing in the magnetic field, sterilization effect was poorer than bigger flow rate. One of the possible reasons is relative with "time window effect". So, under or over the best flow rate 26ml/min, meaning that the time is out of "time window", sterilization effect was poor. Another possible reason is that lower flow rate can not lead sample enough inordinate, so the purpose of overcoming the magnetic field non-uniformity cannot be realized.

Temperature is an important factor affecting sterilization effect of the pulsed magnetic field (Ma Haile, Wu Qiongying, Gao Mengxiang, & Chu Jinyu, 2004). A little raise of temperature under 12°C can significantly improve sterilization effect of the pulsed magnetic field. However, the temperature of static-tube-tests (10°C) was higher than dynamic-tube-tests (6°C), the minimum survival rate for dynamic-tube-tests (0.8%) was lower than that of static-tube-tests (1.58%) (see

Figure 5 for *S. cerevisiae*) by contraries, indicating that effect of dynamic sterilization is superior to static sterilization, this is because that the flow bacteria had more opportunities exposing to area with high intensity of magnetic field in chamber than static bacteria, avoiding appearance of "dead corner".

4. Conclusion

Inconsistency of PMF sterilization is due to well-regulated fluctuation of sterilization effects with magnetic field intensity and pulse number. This phenomenon can be described by window characteristics which is one of the non-thermal biological effects of magnetic fields. The sterilization effect of dynamic-tube-tests is better than one of static-tube-tests.

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